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To cite this article: Halifah Pagarra *et al* 2019 *J. Phys.: Conf. Ser.* **1244** 012022

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Design Two-Level Factorial to Screening Factors Influencing of Pectin Extraction from Banana Peels (*Musa paradisiaca*)

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Abstract. A study was conducted to vary the parameters affecting the production of pectin extraction from kepok banana peels using 2-level factorial design (2LFD). The experimental design was performed to screen or significant environmental factors for the yield of pectin. Factors involved consist of pH, temperature and extraction time. Independent variables are pH (1.5 to 2.5), temperature (60°C to 100°C) and extraction time (60 minutes to 90 minutes). The results showed that the pectin extract produced ranged from 3.46% to 18.31%. Analysis variance (ANOVA), found the two factors that influence very significantly affected by pH and temperature, the interaction between pH and temperature; temperature and extraction time; interaction pH, temperature and extraction time. Statistical analysis shows that the linear model is significant with the value of R^2 is 0.9763. There are four significant factors that influence the pectin yield obtained by 18.31% with a P value less than 0.05 ($P < 0.05$).

Keywords: banana peels, extraction, pectin, two-level factorial design

1. Introduction

Bananas (*Musaceaea* sp.) are Indonesia's leading fruit commodities. Banana production is increasing every year. The amount of banana production in Indonesia in 2015 amounted to 7,299,266 tons [1]. As the increase in banana production will certainly be followed by an increase in banana waste such as the peels and bunches of bananas. The utilization of banana waste is still not optimal. The banana peel waste can reach 40% of the total fresh fruit. Likewise with the waste of banana bunches. Banana peels waste is only used as animal feed; bunches waste only becomes organic waste whose presence can pollute the environment. Banana peels waste can be processed into pectin which has added value with the appropriate method. Thus environmental problems can be reduced [2]. As explained by Wachirasiri et al. [3], that a municipal landfill is a place for banana skin waste disposal that



contributes to existing environmental problems. However, this can be overcome so that the waste is potentially high as a functional food preparation by fiber fractionation method.

Bananas are healthy and nutritious fruit containing 75% moisture, 23% carbohydrates, 1% protein and 0.5% fat [4]. Extraction is the most important process in pectin production. Pectic substances are usually extracted by chemical or enzymatic methods, with physical processes and several chemical stages, which involve hydrolysis, extraction and solubilization macromolecules [5]. In recent studies, The extraction of pectin from fruit peels using a weak organic acid such as citric acid has been intensively conducted [6]–[8]. The extraction of pectin involves the hydrolysis of insoluble protopectin into soluble pectins and then leaching them out of fruit tissues [9].

Pectin is one of the major components of the primary cell wall, and it is generally thought to account for about one-third of all primary cell wall macromolecules. Pectins are a group of polysaccharides consisting mostly of D-galacturonic acid, and Some of the carboxylic groups of galacturonic acid molecules in the pectin chains are methyl esterified, and the percentage of esterified groups is expressed as DE (degree of esterification). Depending on the degree of esterification, pectin is divided into two major groups; higher-ester pectin with DE more than 50%, and low-ester pectin with DE lower than 50% [10]. The yield and quality of pectin depend mostly upon the source, as well as the method employed for extraction of pectin. The yield of pectins extracted from sugar beet by acid (HCl or HNO₃) at varied pH (1–3), temperature (75–90°C) and time (30–90 min) exhibited a galacturonic acid content and an extraction time varying from 295 to 528 mg g⁻¹ (dry weight) and 34% to 94%, respectively [11]. This work aims to extract pectin from kepok banana peels.

2. Research Methods

2.1 Material preparation for banana peels extract

The selected banana peel from ripe banana with yellowish green color fresh kepok banana. Furthermore, the peels part used as the experimental material in this study. Kepok banana peel is cut into small pieces and then washed and dried in an oven at 60°C for five days until the dry weight of the leaves is stable. This dry sample of banana peel is ground and filtered to get a small particle size using a 1 mm filter.

2.2 Pectin extraction

Modification extraction methods from [11][12]. A total of 10 g of kepok banana peel flour was added to aquadest with a ratio of 1:20. Determination of pH 1.5 and 2.5 with 0.5 N HCl solvent, then incubated at 60°C and 100°C for extraction time of 60 minutes and 90 minutes. Stirring is carried out during incubation. After incubation, filtering is done to get the filtrate using filter paper. Furthermore, the filtrate was precipitated with 95% ethanol concentration for 24 hours. The precipitate is washed twice also with 95% ethanol. The obtained pectin is dried in an oven at 50°C until a constant weight is reached. The dried is calculated as the weight of dry pectin (g) per 100 g of the dried kepok banana peel.

2.3 Experimental design and statistical analysis

Factors influencing the extraction of pectin were screened using a 2-level factorial design created by Design Expert software (State-Ease Inc., Statistics made easy, Minneapolis, MN, USA, Version 6.0.4). Variables that have the most significant effect on the yield of pectin extraction were then identified using 2LFD. This design contains a total of 24 experiments. Each independent variable was investigated for duplicates at high (+1) and low (-1) levels. The variables used were pH (A), temperature (B), extraction time variables were coded as -1 (low coded) and +1 (high coded) (Table 1). Variables that have a large effect on the yield of pectin extraction were identified based on a confidence level above 95% ($P < 0.05$). Significant factors and interactions identified from the half-normal plot analysis were chosen to produce a first-order model for texture response after effects and

interactions were evaluated. The significance of the linear effect of the three variables was evaluated by analysis of variance. The coefficient of determination R^2 (pronounced R-Square) and the Adjusted R^2 coefficient are used to evaluate the suitability of the model. The statistical significance of the second-order model equation is determined by a significant F value and not significant-of-fit F-value [13].

Table 1. The range of variable and their coded levels independent

Factor	Name	Low coded	High coded
		(-1)	(+1)
A	pH	1.5	2.5
B	Temperature (°C)	60	100
C	Extraction Time (min)	60	90

3. Result and Discussion

2LFD is used as a screening method to determine which of the three variables that most significantly affect the results of kepok banana peel extract. This variable consists of pH, temperature and extraction time and the design consists of 24 experiments. The filtering design is used to detect factors or independent variables that have a higher impact on the variable response results of kepok banana peels extraction.

Table 2. Analysis of Variance (ANOVA) for exo-polygalacturonase activity using 2LFD

Source	Sum of squares	Degrees of freedom	Min square	Value > F	P>F
Model	683.29	7	97.61	94.01	< 0.0001*
A	23.21	1	23.21	22.35	0.0002
B	568.43	1	568.43	547.44	< 0.0001
	3.42	1	3.42	3.29	0.0883
AB	39.53	1	39.53	38.07	< 0.0001
AC	0.36	1	0.36	0.35	0.5641
BC	20.06	1	20.06	19.32	0.0005
ABC	28.30	1	28.30	27.25	< 0.0001
Pure Error	16.61	16	1.04		< 0.0001
Cor Total	699.91	23	97.61		
$R^2 = 0.9763$					
Adj R-squared = 0.9659					
Std. dev.= 1.02					

Table 2 shows the predicted level of kepok banana peel extraction from experimental data. A normal half plot can be used to determine significant factors that affect response. In the study of the yields of kepok banana peel extract extraction, the experimental design began with a screening experiment involving three factors which literature review had a high impact on enzyme production. In this review, there are six factors that have been studied using 2LFD experiments as shown in Table 2 and the value of yields of kepok banana peel extraction is predicted by mathematical models and experimental data. Interpretation of the research was statistically analyzed using analysis of variance (ANOVA), and complete yields are shown in Table 2. The probability values (P-values) of each term and factor interactions are listed in Table 3. The P-value less than 0.005 means that the factors this is significant to the responses studied. This is based on a confidence level set at 95%. This model is

significant with a probability of <0.0001 . This means that the regression model was produced to explain the correlation of yields of kepok banana peel extraction with the factors tested exactly in statistics. Table 2 Analysis of variance (ANOVA) for the yields of kepok banana peel extraction using a two-level factorial design. pH is one of the most important and very significant variables obtained from the yields of statistical analysis through this study. Temperature is also the next significant factor identified in this statistical analysis. The importance of temperature in the development of biological processes is very clear as the temperature can determine the effects of kepok banana peel extraction yields. According to Chan et al. [14], that the yields of cocoa peel pectin extraction with HCl solvent showed the highest pectin yield at pH 2.5. Similarly, the extraction temperature is very influential, where the extraction temperature of 50°C - 95°C using citric acid at pH 2.5 or 4.0 and Hydrochloric acid at pH 2.5 or 4.0 increased significantly ($p < 0.05$) of pectin yield.

Two-level factorial design studies have been used to filter out significant factors affecting the results of pectin extraction of banana peels. The selected and filtered factors include pH, temperature, and time of extraction. The performance of pectin extraction was studied regarding pectin yield. The results of this experiment suggest that the proposed mathematical model can explain the capabilities of the factors studied with the determination coefficient at 0.9763 ($R^2 = 97.63\%$). Two variables tested were significant factors influencing pectin yield obtained 18.31% with a P value less than 0.05 ($P < 0.05$).

A half-normal plot is used to determine the significant factors affecting the response in this study. This plot determines the absolute range of factor impression and shows the symbols of factors far below the linear line is a significant factor in the response [15]. In Figure 1, a half-normal plot indicates that the factor symbols far from the linear line are a significant factor in the yields of pectin extract. The effect of A (pH), B (temperature), is located far from the line and thus shows a strong signal. This decision is related to P-value < 0.0001 (Table 2). The next significant factor was the interaction factor between pH and temperature (AB factor), the interaction between temperature and extraction time (BC factor), the interaction between pH, temperature and extraction time (ABC factor).

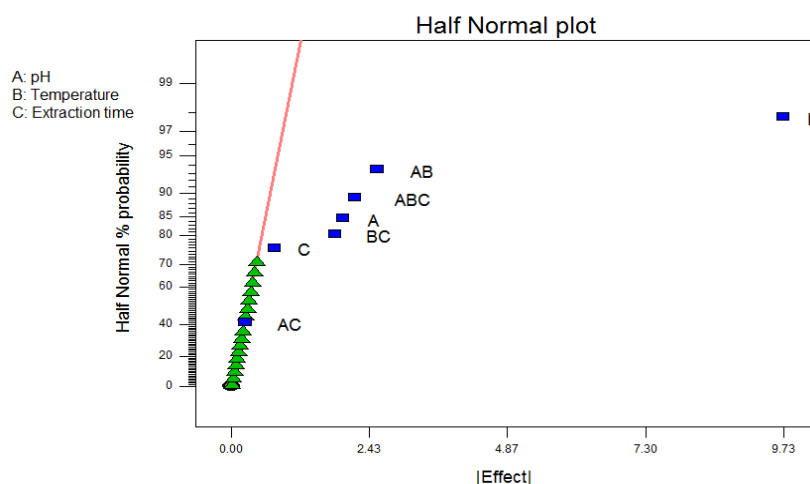


Figure 1. The plot is half normal for the effect of pH (A), temperature (B), extraction time (C) of pectin yield

Responses to every main factor and also the interaction between factors are also examined statistically and also patents the response that these factors are also known to be certain in response. In this study, as shown in Figure 1, there were two main factors which gave a very statistically significant effect on the results of banana peel extract, A (pH) and B (temperature). Also, the C factor (extraction

time) was found to be insignificant, i.e., statistically it did not give an impression to the extraction process than the results of banana peel pectin in this study.

Figures 2 show responses that this major factor is important in the results of extraction of banana peel pectin. Instead of Figure 2a and 2b, it can be seen that the increase in extraction activity factors in rank (-1 and +1) studied has given an insignificant effect on the results of pectin extract. However, an increase in the value for pH and temperature in the position studied has led to an increase in the results of the pectin extract or said to have given a significant impression of the response studied.

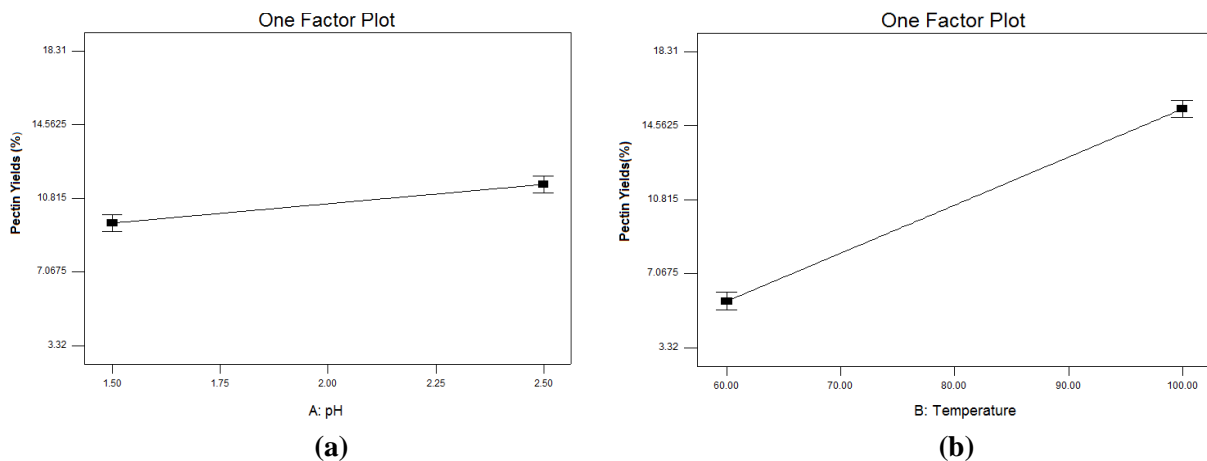


Figure 2. (a) Plot the main effect A (pH) on the results of banana pectin extract; (b) Plot the main effect B (temperature °C) on the results of banana pectin

In addition to the main factor impacts, the interaction between factors also shows different effects as a result of the statistical analysis that has been carried out. Figure 3a shows the interaction effect between 2 significant factors on the extraction of banana peach pectin. Interaction AB (pH with temperature), BC interaction (temperature with extraction time) has a significant effect on the responses studied with P-value <0.0001. Fig 3b, also shows the effect of three ABC factors (pH, temperature and extraction time) also has a significant effect on the response with P-value smaller than 0.05. Meanwhile, the effects of C and AC, having P value above 0.05 are not significant factors. The interacting effect on Figure 3a shows a non-crossed line, and the line is not parallel, this interaction is called ordinal interaction. If the line is parallel, there is no interaction effect [16].

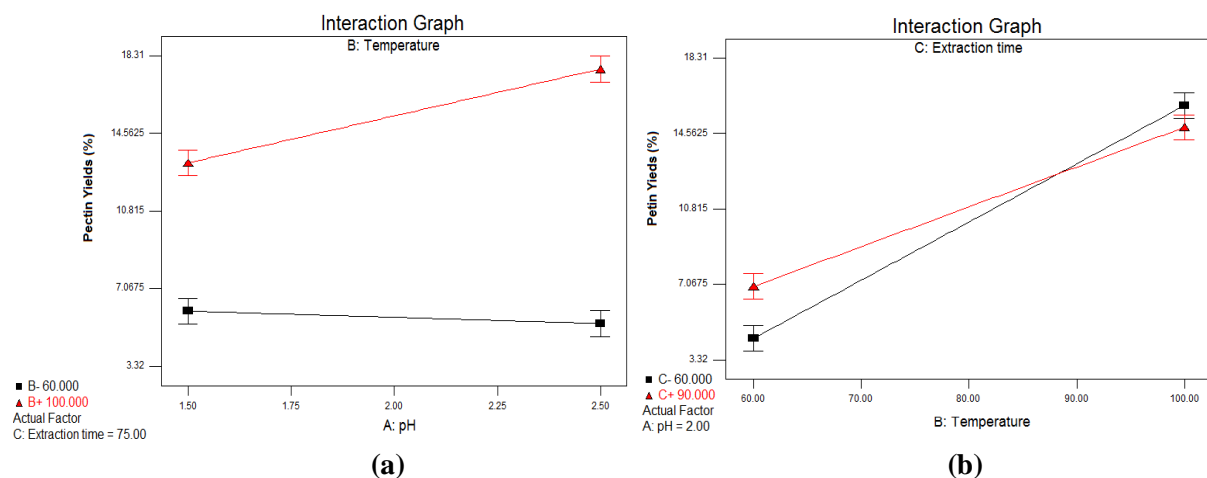


Figure 3. (a) Plot significant interaction factors between pH and temperature on the results of banana pectin extract (%); (b) Plot significant interaction factor between temperature and extraction time on yields of banana pectin extract (%)

Figure 3a shows the first interaction between A (pH) and B (temperature). The interaction effect of AB has the greatest interaction, where effect A is greater when B+ is at a temperature of 90°C. This plot shows that when the data is bound to B-, the effect is flatter than B+. However, in this study, the target is the result of pectin extract. Therefore, B+ is the best choice based on the target. This interaction may occur because both factors contribute most to the pectin yield compared to other factors. Figure 3b shows the last interaction between two factors, temperature (B) and extraction time (C). The slope for high concentration C+ (extraction time of 90 minutes) and low concentration of C- (extraction time of 60 minutes) to both steep. Figure 3a shows the first interaction between A (pH) and B (temperature). The interaction effect of AB has the greatest interaction, where the effect of A is greater when B+ is at 90°C. This plot shows that when the data is bound to B- the effect is flatter than B+. However, in this study, the target was the result of pectin extract. Therefore, B+ is the best choice based on the target. This interaction can occur because both factors contribute most to the results of pectin compared to other factors. Figure 3b shows the last interaction between two factors, temperature (B) and the extraction time (C). Slope for high extraction time C+ (extraction time 90 minutes) and low extraction time C- (extraction time 60 minutes) showed both steep at pH 2. But at C- (60 minutes) there was an increase in the results of pectin extract with an increase in temperature from 60°C to 100 C and the result is steeper (higher) than in C+ (90 minutes).

4. Conclusion

The effect of the operating parameters on the results of pectin has been evaluated using a full two-level factorial Design. From experimental studies, the pH factor (A) and temperature (B) are identified as the most significant factor of pectin yield three interactions between AB, BC, and ABC affect one factor to another in the pectin extraction process. The combination effect between A and B shows the highest percentage pectin yield contribution. Therefore, it can be concluded that the formation of pectin depends on this two factors. Therefore, pH and temperature are those significant factors and are used in subsequent optimization experiments using a centralized composite design (CCD). The screening of this factor was aimed at identifying significant factors and subsequent research using RSM was to use these significant factors to obtain optimum pectin yields in the studied stages.

Acknowledgments

The authors are grateful and would like to express their sincere gratitude to the Universitas Negeri Makassar (UNM) which facilitates research funding from DIKTI and Department of Biology, Faculty of Mathematics and Natural Sciences, for their kind support.

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